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Europäisches Patentamt
European Patent Office
Office européen des brevets

11 Publication number:

0 251 494
A2

12

EUROPEAN PATENT APPLICATION

21 Application number: 87304796.3

51 Int. Cl.4: A61K 49/02 , A61K 43/00 ,
A61K 47/00

22 Date of filing: 29.05.87

30 Priority: 23.06.86 US 877327

43 Date of publication of application:
07.01.88 Bulletin 88/01

64 Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

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54 System for administering therapeutic or radiodiagnostic compound.

57 A system for localizing a diagnostic or therapeutic agent to an internal target site. The system includes (1) a biotinylated compound, (2) an avidin-containing binding protein capable of localizing selectively at the target tissue, when administered parenterally, and (3) a clearing agent which can bind to and cross-link the binding protein, to form a protein aggregate which is readily cleared from the subject's bloodstream. In practicing the method of the invention, the binding protein is administered to the subject parenterally, and allowed to localize at the target site, typically within 1-4 days. This is followed by a chase with the clearing agent to remove circulating, but not target-localized binding protein. When the biotinylated compound is administered, binding of the compound to the localized binding protein, and rapid clearance of unbound compound by the kidneys, results in selective localization of the compound at the target site.

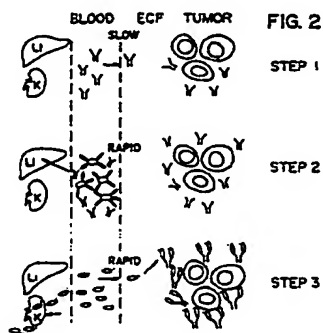


FIG. 2

STEP 1

STEP 2

STEP 3

SYSTEM FOR ADMINISTERING THERAPEUTIC OR RADIODIAGNOSTIC COMPOUND

The present invention relates to a system for administering a therapeutic or diagnostic compound, and in particular, a radionuclide, to produce target-specific localization of the agent.

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A major focus of current drug research is to improve drug targeting to internal target sites, such as to
 25 solid tumors or specific organs. The objective of drug targeting is to enhance the effectiveness of the drug by concentrating it at the target site, and minimizing its effects in non-target sites. For example, where the drug is used for therapeutic purposes, such as to treat a solid tumor, drug targeting allows more effective dosing at the target site with fewer non-tumor related side effects. Similarly, where the drug agent is a radionuclide for use in radioimaging, targeting gives enhanced contrast between the target and background
 30 areas, because of reduced background levels of the radionuclide.

Radionuclides are an important group of pharmaceutical agents for which a variety of targeting strategies have been proposed. Included in this group are radioimaging compounds, such as metal chelates of ^{111}In , ^{67}Ga , ^{64}Cu , ^{99}Tc , ^{68}Ga , ^{62}Zn , ^{67}Cu , ^{197}Hg , ^{97}Ru , ^{57}Co , or ^{53}Co , which are used to image internal sites,
 35 particularly solid tumors, by intravenous administration and systemic uptake of the chelates. Also included are radiotherapeutic agents, such as the metal chelates of ^{90}Y , ^{197}Hg or ^{67}Cu , or conjugates of other radioactive elements, such as ^{131}I which are used in treating tumors and the like, based on localized cell destruction from ionizing radiation. A related group of pharmaceutical agents are non-radioactive metal chelates such as iron, copper or ruthenium chelates, which produce cytotoxic effects through redox mechanisms, and can also potentiate the cytotoxic action of radiation on cells.

Previously, the inventors have described several novel chelate compounds which are useful for
 40 targeting radionuclides and radiosensitizing metals to internal sites, particularly solid tumors. In general, these compounds are bifunctional chelating agents which have, as one functional group, a chelating moiety capable of forming a tight complex with a metal ion, and as a second functional group, a chemically reactive moiety, such as a nitro or amine group, through which the compound can be coupled to a targeting
 45 or other molecule (Meares, 1976; Goodwin et al, 1975, 1976, 1979).

One novel class of chelate compounds which has been developed by the inventors are various ethylenediaminetetraacetic acid (EDTA) chelates of bleomycin, which is an anti-tumor antibiotic which localizes within many types of tumors (Umezawa, Fujii). The bleomycin/EDTA compounds have been shown to give selective tumor localization of a variety of radionuclides, including ^{57}Co and ^{111}In , in solid tumors.
 50 One of the earliest of these compounds was prepared by alkylating purified bleomycin A_2 with a reactive bifunctional compound, such as p-bromoacetamidobenzyl-EDTA (BABE-EDTA), to link the chelate to bleomycin through a sulfonium group (DeRiemer; Goodwin, 1979, 1981; Chang). A more recent compound, formed by joining a bifunctional EDTA molecule to a bleomycin-Co complex through a monodentate cobalt-sulfur coordinate bond, is described in co-owned U.S. Patent Application for "Bleomycin Conjugates and Methods", Serial No. 712,377, filed March 15, 1985.

One of the limitations which has been observed in targeting small radionuclide compounds, such as the above bleomycin/metal chelate compounds, to a target site, such as a solid tumor, is relatively low concentration of the compound at the target site. The low drug dose at the target site is due to the rapid clearance of the compound by the kidneys, which limits the amount the compound in the bloodstream available for localization at the target site. Merely increasing the dose of the administered compound is not a practical solution, since most of the radionuclides are toxic and therefore dose-limiting.

One method for increasing the concentration of a dose-limited, but rapidly cleared, target compound is to coadminister the compound in an antibody-complexed form. Because of its relatively large size, the complex is not cleared by the kidneys, but instead, is removed slowly from the bloodstream over a several day period by the reticuloendothelial system (RES). This approach has been investigated previously by the inventors, using two monoclonal antibodies (Mabs) prepared against the bifunctional indium/chelate compound L-benzyl-EDTA-¹¹¹In (LBEDTA-In). Binding studies showed that both antibodies were specific for the indium chelate, giving K_b values for the indium chelate which were at least about 20 times those of the chelates of other metals. The antibodies, when coadministered with BLEDTA-¹¹¹In, increased the whole body level of BLEDTA-¹¹¹In after 24 hours between 10-30 times, presumably by retaining the BLEDTA-In compound in a tightly bound systemic form which is cleared slowly from the bloodstream.

The increased uptake of the compound in the presence of circulating anti-compound antibodies is, however, a relatively non-specific effect, since a variety of organs which were tested for ¹¹¹In levels also showed significantly increased radioactivity after 24 hours. Therefore, the advantage of enhanced tumor uptake of the compound produced by coadministration of an antibody is partially offset by (1) higher background levels of radioactivity (or a therapeutic agent) in non-tumor organs, and (2), greater total patient exposure to the conjugate, e.g., greater radiation exposure in the case of a conjugate having a chelated radionuclide.

Although it may be possible to reduce these unwanted side effects by flushing the patient's bloodstream with a non-toxic or non-radioactive competing antigen, the improvement in terms of reduced exposure to the compound is not dramatic. Earlier studies conducted by the inventors showed, for example, that whole body BLEDTA-¹¹¹In levels are reduced only about 20% three hours after giving a flushing dose of Fe-EDTA. Further, the antibody-enhancement approach just described requires periods of at least several hours for significant target distribution effects; therefore, the method is not suited to radionuclides such as ^{99m}Tc and ⁶⁸Ga which have half lives of between about one to a few hours.

The system of the invention includes (a) a biotinylated compound containing the agent to be localized (b) an avidin-containing protein capable of localizing selectively at a target site when administered to a subject parenterally, and (c) a clearing agent capable of reacting with the binding protein when circulating in the bloodstream of the subject, to form a macromolecular aggregate which can be cleared by the subject's reticuloendothelial system.

The individual components of the system for use in diagnosis or therapy using the above system are included in the invention.

The present invention is further described by way of example only with reference to the accompanying drawings, in which:

Figure 1 shows the molecular structure of a biotinylated EDTA-metal chelate compound designed for use in the invention;

Figure 2 illustrates, in diagrammatic form, the steps by which a therapeutic or diagnostic compound is localized in a tissue, according to the method of the invention and in the Figure "Li" denotes the liver and "K" denotes the kidneys; and

Figure 3 shows whole-body photo-emission scans of a tumor-bearing animal 3 hours (A) and 24 hours (B) after administration of a radionuclide-chelate compound.

I. Preparing the System Components

A. Biotinylated Compounds

The invention is designed for use in targeting a therapeutic or diagnostic agent at a specific internal body site, such as a solid tumor or selected organ or tissue site. The agent which is targeted is a biotinylated compound having a pharmaceutically active therapeutic or diagnostic, particularly radiomaging, moiety (the active moiety), and one or more biotin moieties which can bind specifically and with high affinity to an avidin-containing binding protein.

The active moiety of the compound is a pharmaceutically active agent, such as a drug, radionuclide, hormone, toxin, metabolite, anti-metabolite, vitamin, enzyme-cofactor, or the like which can be derivatized to one or more biotin groups without loss of activity. Such active agents include anti-tumor agents, as exemplified by doxorubicin, cisplatin, DNA alkylating or cross-linking agents, and antimetabolites; and antimicrobial agents, such as aminoglycosides and polyene and macrolide antibiotics, such as amphotericin B. One major class of therapeutic and diagnostic agents useful in the invention are chelated metals, including chelated radionuclides useful for radioimaging, such as ^{111}In , ^{67}Ga , ^{64}Cu , $^{99\text{m}}\text{Tc}$, ^{62}Zn , ^{67}Cu , ^{167}Hg , ^{97}Ru , ^{57}Co , or ^{53}Co ; chelated radionuclides useful for tumor therapy, such as ^{90}Y , ^{197}Hg or ^{67}Cu ; and a radio-sensitizing chelated metals, such as chelated iron, copper or ruthenium.

The method of the invention requires that the biotinylated compound be sufficiently small and soluble when administered parenterally that it can be cleared rapidly from the kidneys. Typically molecules with molecular weights less than about 50,000 daltons, and preferably less than about 10,000 daltons, and which exist predominantly in monomolecular form in serum generally satisfy these requirements.

Methods for derivatizing small target molecules with one or more biotin groups follow well-known coupling procedures for biotinylating compounds at reactive chemical sites, such as carboxyl, amino, nitro, hydroxyl, aldehyde, and sulhydryl sites. In one preferred method, the N-hydroxysuccinimide ester of biotin (biotin-NHS), a commercially available compound, is reacted directly with an amine group in the targeting compound, for coupling through an amide linkage. The amine group may itself be added to the target compound as a spacer arm, which is also joined to the target compound by standard coupling techniques. By way of illustration, the biotinylated EDTA compound illustrated in Figure 1 was formed by reacting a putrescein (1,4-butanediamine) with p-isothiocyanato benzyl EDTA, to couple the putrescein spacer arm to the bifunctional EDTA compound. Derivatization with biotin-NHS then yielded the desired biotinylated compound. Other coupling methods suitable for producing biotinylated target molecules are available and known to those in the art.

Biotinylated compounds containing two or more biotin groups, to give enhanced binding to avidin or streptavidin, may be prepared by the same general methods discussed above. In derivatizing an active agent with multiple biotin groups, the individual groups can be attached to different sites on an active agent, or may be attached at different positions on a single spacer arm attached to the active agent.

B. Avidin-Containing Binding Proteins

The avidin-containing binding protein in the system of the invention serves both as a targeting agent capable of localizing specifically at an internal target site, and as a binding agent, for binding the biotinylated compound to the target site.

The inventors have earlier described a targeting system in which the binding protein is an antibody capable of binding specifically to a hapten-derivatized compound. Whereas antibodies offer the advantage that they can be prepared against a wide variety of epitopes, avidin has the advantage of a very high binding affinity (about 10^{15} M^{-1}) for the epitope biotin, as compared to binding affinities of between about 10^9 to 10^{12} for hapten/antibody pairs. The advantage of the high binding constant in the present invention is that the invention can be practiced using much smaller quantities of injected binding protein. Specifically, the amount of avidin which need be injected can be several orders of magnitude smaller than the amount of antibody needed to produce equivalent levels of bound target compound at the target site. Accordingly, immune reaction problems which may arise by parenteral administration of relatively large doses of foreign protein are greatly minimized.

Avidin, an egg protein, and streptavidin are commercially available in purified form suitable for use in human therapeutics. These proteins can be derivatized to antibody or antibody fragment molecules by known coupling methods, which include condensation and cross-linking reactions. The monoclonal antibody/streptavidin conjugate described in Example 2 was formed using the cross-linking reagents 2-iminothiolane and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, according to known methods. Other coupling agents, such as soluble carbodiimides, and di-N-hydroxysuccinimide compounds may also be used.

Considering the targeting function of the binding protein, the invention relies on the ability of the protein, when administered parenterally, to localize selectively from the bloodstream to the target site. For efficient localization, this requires that the protein (a) have a relatively long circulating half life in the bloodstream, and (b) be capable of accumulating in the target organ. In terms of size requirements, avidin, streptavidin,

and antibodies or antibody fragments derivatized with either is large enough to prevent rapid renal clearance, but not so large as to promote rapid clearance by the RES. Binding proteins, including various types of hybrid proteins discussed below, in the size range between about 50-500,000 daltons are most suitable.

5 The targeting capability of the binding protein may be based on the ability of the protein to bind specifically to target site antigens, or on protein size or membrane permeability characteristics, or to a combination of these factors. Most target sites, including tumor target sites contain tissue-specific surface antigens against which the binding protein can be directed, and antibodies specific against a variety of normal and malignant tissues, such as used in Example 2, have been reported. The antibody may be an intact serum, or preferably monoclonal, antibody specific against target tissue antigens, or an antibody fragment, such as the F(ab')₂ or Fab fragment produced by enzymatic cleavage of intact antibody. In the antibody-avidin conjugate, the antibodies or antibody fragments in the hybrid protein would function is tissue targeting, while the avidin moiety would provide high affinity binding for biotin-labelled compounds which are targeted to the site.

15 In another embodiment which is suitable for tumor targeting, selective protein localization in the tumor is based on the ability of the protein to penetrate selectively the more permeable, i.e., leaky, capillaries which normally are associated with solid tumors. The advantage of this approach is greater simplicity in preparing the binding protein.

Examples 1 and 2 below illustrate tumor targeting with streptavidin alone (Example 1), and streptavidin conjugated with an anti-tumor monoclonal antibody.

C. Clearing Agent

25 The clearing agent in the system of the invention functions to bind to and cross-link binding proteins which are circulating in the bloodstream of the treated individual, to promote rapid clearance of the binding protein aggregates by the RES. The agent is preferably large enough to avoid rapid renal clearance, and contains sufficient multivalency to cross-link and aggregate circulating binding proteins. Therefore, the protein preferably has a molecular weight of about 50,000 daltons or more, and is derivatized with biotin at a mole ratio of about 1:4 protein:biotin or higher. Here it is noted that protein aggregation by the clearing agent also requires at least two avidin molecules on each binding protein.

One preferred clearing agent, for use in humans, is a human protein derivatized with multiple biotin groups. For example, human transferrin can be biotinylated by reaction with biotin in the presence of a suitable condensing agent, such as a soluble carbodiimide, or by reaction with the above biotin-NHS reagent.

II. Therapeutic and Radiodiagnostic Methods

A. Localizing the Binding Protein

The method of the invention is designed for targeting a therapeutic or radiodiagnostic agent to a selected internal body site, such as a tumor region, an internal organ, or some other specific tissue region.

45 As a first step in the method the binding protein is administered parenterally, i.e., into the bloodstream, preferably by intravenous (IV) administration. From here, the protein slowly passes from the blood, through the extracellular fluid (ECF), and into the body tissues, including the target site, accumulating preferentially in the tumor site because of the targeting feature of the protein. This initial step is illustrated at the top in Figure 2, which shows slow passage of an avidin-labeled antibody binding protein from the bloodstream (dotted-line column) through the ECF into a tumor site.

50 According to an important advantage of the method, the binding protein is delivered in non-complexed form, i.e., without bound biotinylated compound so that the treated individual is not exposed to the compound during the extended period of protein localization at the tumor site. The amount of binding protein which is injected is calculated to yield a selected concentration of the biotinylated compound at the target site, in the final targeting step. This amount will depend on several factors which include: (a) the relative degree of localization of the binding protein in target and non-target regions of the body, (b) the persistence of the binding protein in the blood and, most importantly, (c) the binding constant of the binding protein, when localized at the target site, for the epitopic compound.

The optimal amount of binding protein which is administered can be approximately determined by combining known binding constant data with empirical studies on animal model systems. Studies conducted in support of the present invention indicate that effective targeting of a biotinylated EDTA compound required an initial avidin or streptavidin doses which are substantially less than the 10-100 mg dose required for achieving the same level of localized chelate using an anti-chelate antibody as the prelocalizing agent.

The administered binding protein, which has a circulating half life in the blood of one to several days, is allowed to localize in the target organ over period of typically about 1-4 days. During this period the binding protein is slowly taken up from the blood by the target tissue, as well as other tissues. In the case of tumors, binding protein accumulation at the target site is enhanced because of the relatively greater leakiness of the capillaries which supply the tumor. As mentioned earlier, tumor localization can be based solely on preferential protein (e.g., avidin or streptavidin) leakage into the tumor. This mechanism is illustrated in the targeting methods illustrated in Examples 1 and 2, which both show accumulation of an avidin binding protein in tumor regions 24 hours after intravenous administration.

B. Clearing the Binding Agent

In the second step of the method, the circulating binding protein is cleared rapidly from the bloodstream, to reduce total blood levels of the protein severalfold, without appreciably effecting the levels of binding protein which have accumulated in the target. This step, which is illustrated in the center frame of Figure 2, is based on the formation in the bloodstream of aggregates of binding proteins and clearing agent, and rapid clearance of these aggregates by the RES. In the figure, the aggregates formed in the bloodstream are shown being removed by the liver, the principle site of uptake by the RES. Because of its rapid removal, the clearing agent does not accumulate appreciably outside the bloodstream, and therefore has little effect on the disposition of binding protein already localized outside the bloodstream.

The amount of clearing agent which is administered is preferably in a molar ratio of between about 1:5-5:1 with respect to the quantity of binding protein calculated to be the bloodstream of the treated individual at the time of the clearing step. Experiments conducted in support of the application indicate that the amount of binding protein present in the bloodstream 24 hours after iv administration is typically between about 15-25% of the total amount administered.

The total time allowed for clearance of the binding protein, i.e., before the epitopic compound is administered, may be as short as 15 minutes, but typically ranges from about 1-4 hours. In the study reported in Example 2, blood levels were reduced about over 40 fold with the chase, as measured by the amount of radiolabelled biotinylated chelate taken up by the blood before and after the clearing step.

C. Uptake of the Biotinylated Compound

In the final targeting step, the biotinylated compound is administered parenterally, and preferably intravenously, for selective uptake of the localized binding protein. This step, which is illustrated at the bottom in Figure 2, involves rapid uptake of the compound by the localized binding protein, in competition with rapid clearance from the bloodstream by the kidneys.

The amount of biotinylated compound which is administered is calculated to produce a desired concentration of compound at the target site shortly after compound injection, usually within 1-4 hours after injection. As with the binding protein, the optimal dosage can be calculated approximately from studies on a model animal system combined with the known binding affinity of the binding protein for the compound.

A number of studies on the tumor localization of biotinylated chelate-radionuclide compounds have been carried out in support of the invention, and some of these studies are detailed in Examples 1 and 2. With both streptavidin and antibody-streptavidin conjugate binding proteins, tumor/blood ratios increased severalfold with administration of the chase.

In a related study, detailed in Example 3, a monoclonal antibody (not conjugated to avidin) specific against a non-biotinylated EDTA chelate was administered, followed by a chase after 20 hours with transferrin-labelled chelate. One hour later, chelate-⁵⁷Co was given, and allowed to localize for three hours. Whole-body radioimaging of the treated animal gave the results seen in Figure 3A, which shows localization of the label in the kidneys (K), bladder (B) and flank tumor (T). The distribution of the label in

the tumor, blood and other tissues three hours after label injection is shown in Table 1 in Example 3. The data show high tumor-to-organ ratios for blood, and other internal organs. The higher levels of radiolabel in the kidneys seen in the present study is due presumably to slower clearance of the radiolabeled compound from the kidneys.

Figure 3B shows the same animal imaged in Figure 3A, but imaged 24 hours after compound administration. The major difference in the longer-term imaging is an absence of label in the bladder. Good tumor imaging with low background is still achieved. The example 3 study indicates the enhancement in tumor imaging that can be achieved by the method of the invention, but differs from the invention in that the antibody (binding protein) levels which must be administered are substantially higher than those needed in the present invention to achieve sufficient concentration of the targeting compound in the target site.

From the foregoing, it can be appreciated how various objects and features of the invention are met. The method provides selective localization of a therapeutic or radioimaging compound, based on accumulation of a target-specific binding protein at the selected target site. This feature allows for improved therapeutic effect with reduced side effects for therapeutic agents, and improved imaging with reduced background levels, particularly background levels due to circulating radionuclide.

Because the localization of the compound occurs shortly after compound injection, e.g., 1-4 hours, radionuclides with half lives of about 1-6 hours can be used for radioimaging. In particular, the method allows both ^{99m}Tc , whose half life is about 6 hours, and ^{68}Ga , whose half life is 68 minutes, to be used at relatively low radioactivity levels for radioimaging of tumors or other internal target sites. The particular advantage of ^{68}Ga as a radioimaging agent is its use in photon emission tomography, an imaging technique that allows quantitation of localized radiolabel and resolution down to 5 mm. Heretofore, the large amount of radionuclide needed for imaging required on on-site cyclotron. With the present invention, the ability to localize large quantities of the radionuclide in an hour or less is compatible with radionuclide amounts which can be produced with a much less expensive ^{68}Ga generator.

The rapid clearance of non-localized compound by the kidneys means that the toxicity associated with the compound in systemic form can be reduced substantially. In the case of ^{111}In radioimaging, for example, it has been necessary heretofore to attach the metal to a circulating protein, to achieve high levels of the radionuclide at a localized site. This approach is limited by the amount of protein-conjugated metal which can be safely administered due to high levels of the radionuclide in the bloodstream over a several day period. In the present invention, most of the non-localized radionuclide is cleared in a few hours. This advantage also applies to therapeutic drugs, such as anti-tumor drugs, which must be given in large doses to achieve an effective drug level at the target site. Drug treatment has been limited heretofore, for many anti-tumor drugs, by serious side effects associated with large dose levels. In the present invention, the specific binding of the drug at the target sites, due to the presence there of concentrated binding protein, allows therapeutically effective drug concentrations to be achieved at the tumor site with smaller doses of injected drug.

According to another important feature of the invention, the avidin-containing binding protein can be administered in relatively low doses due to its high binding affinity for the biotinylated target compound. Therefore the risk of immune reaction to the administered binding protein is substantially less than where the binding protein is an unconjugated antibody, which must be administered in substantially greater dosage. Further, the cost of treatment materials is reduced.

The following examples illustrate the preparation and use of specific embodiments of the invention, but are in no way intended to limit the scope of the invention.

Example 1 Biodistribution of In-111 Biotin Compound

Biotin was coupled to p-isothiocyanato EDTA through a putrescein spacer, as described above, and the biotinylated chelate compound was labeled with In-111, according to standard methods. The animals retained about 6% of the total radioactivity 6 hours after intravenous injection, and about 2%, 24 hours after injection. When the In-111 compound (0.03 nmoles) was complexed with avidin (0.1 nmoles), and administered intravenously, 70% was retained after 24 hours, but was localized mostly in the liver (42/g liver; 0.03%/g blood%). When streptavidin was substituted, 75% was retained after 24 hours, but stayed in circulation to a much greater extent (10%/g liver; 6%/g blood

BALB/c mice with KHJJ tumors were pretargeted with 0.25 nmole streptavidin by intravenous injection. At 20 hours, an iv chase of biotinylated human transferrin was given, followed at 21 hours with 0.016 nmole of the above biotinylated EDTA-In-111 compound. The biodistribution of the In-111 compound, as measured 3 hours after compound administration, showed a tumor uptake level of about 0.45% dose/g, and a tumor/blood ratio of about 4.74. In the absence of chase (the biotinylated human transferrin given at 20 hours), the tumor/blood ratio was about 1.52.

The data above indicate that (a) good tumor targeting can be achieved by chelate binding to prelocalized streptavidin, and (b) the removal of blood streptavidin by a chase significantly reduces In-111 background due to blood levels of the bound chelate complex.

Example 2 Targeting with Chimeric Antibodies

Mouse monoclonal antibody SIC5, an IgG2a antibody, which is anti-idiotypic to surface IgM on mouse B cell lymphoma 38C-13 was covalently linked to streptavidin using the bifunctional crosslinking reagents 2-iminothiolane and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, both obtained from commercial sources. Biotinylated EDTA complexed with In-111 was prepared as above.

The bifunctional chimeric antibody was injected intravenously in C3H mice with 38C-13 flank tumors, and allowed to localize for 20 hours, at which time biotinylated human transferrin was given as a chase. One hour later, when the chase had largely cleared the blood of the antibody, the animals were each injected IV with the biotinylated-EDTA-In-111 compound, and organ distribution of radioactivity was measured within 1-3 hours, when renal clearance had lowered the background levels of In-111 sufficiently. Control animals were similarly treated but without receiving the chase and/or antibody, and with covalently labeled In-111 chelate conjugates of the SIC5 antibody.

With covalent In-111 antibody, background was high in all organs, especially liver, spleen, lymph nodes and blood. In pretargeted animals (initial antibody administration), background levels were also high, especially in blood. The chase removed most of the blood background with improved tumor/blood ratios (greater than 6 fold increase).

Example 3 Tumor Imaging

Three A Balb/c mice prepared with a KHJJ adenocarcinoma tumor implants in the flank, were administered about 100 mg of monoclonal antibody specific against an EDTA conjugate. Twenty hours after antibody administration, the animals were given a chase of the transferrin/EDTA-conjugate clearing agent, and one hour later (21 hours after antibody administration), the animal received an injection of the EDTA conjugate complexed with In-111. Three hours later, a whole-body scan, using computer digitation of the whole-body image, was performed on the animals. A representative image obtained for one of the animals is shown in Figure 3A. The image shows a concentration of radiolabel in the kidneys (K), bladder (B), and the tumor region (T). About 16% of the ¹¹¹In administered remained after 3 hours. Two of the animals were sacrificed at this time and examined for ¹¹¹In levels in the blood, tumor, and other tissues listed in Table 1 below. As seen from the table, the regimen gave good tumor uptake, and high tumor to blood and low tumor to kidney ratios, consistent with (a) localization of radionuclide in the tumor, and (b) rapid clearance of the radionuclide from the blood by the kidneys.

TABLE 1

	<u>% Dose Per</u>	<u>Tumor/</u>
<u>Organ</u>	<u>gm Organ</u>	<u>Organ</u>
Blood	0.71	5.55
Heart	0.40	9.85
Lungs	1.01	3.91
Liver	1.24	3.20
Spleen	0.39	10.11
Kidneys	37.78	0.11
Tumor	3.95	1.00
Muscle	0.21	18.52
Bone	1.06	3.73
Skin	0.05	4.64
Gut	1.17	3.39

A similar whole-body scan was made 24 hours later (48 hours after the antibody administration). The image obtained, shown in Figure 3B, is similar to the earlier scan, but shows relative absence of label in the bladder. About 14% of the original ¹¹¹I material injected was present.

While the invention has been described and illustrated with respect to specific embodiments and features, it will be appreciated that various changes and modifications can be made without departing from the invention.

Claims

1. A system for administering a diagnostic or therapeutic agent to an internal target site of a subject, comprising
 a biotinylated compound containing the agent derivatized with biotin,
 an avidin-containing binding protein capable of localizing selectively at the target tissue when administered to the subject parenterally, and
 a clearing agent capable of reacting with said binding protein when circulating in the bloodstream of the subject, to form a macromolecular aggregate which is cleared by the subject's reticuloendothelial system.

2. A system as claimed in claim 1, wherein the binding protein is avidin or streptavidin.

3. A system as claimed in claim 1, wherein the binding protein is an antibody conjugated to avidin or streptavidin.

4. A system as claimed in any one of claims 1 to 3, for use in delivering a radionuclide to the target site, wherein said biotinylated compound is a biotinylated chelate compound complexed with a metal radionuclide ion to form a stable metal chelate complex.

5. A system as claimed in claim 4 wherein the chelate compound is a metal chelate of a 1-phenyl or 1-benzyl EDTA.

6. A system as claimed in claim 5, wherein the epitope-chelate compound is a metal chelate having a thiobutane spacer arm linked to the benzyl moiety.

7. A system as claimed in any one of claims 4 to 6 which is for use in treating a solid tumor and wherein the metal chelate is a chelate of ⁹⁰Y, ¹⁹⁷Hg or ⁶⁷Cu, or which is for use in radiosensitizing a body tumor and wherein the metal chelate is a chelate of iron, copper or ruthenium.

8. A system as claimed in any one of claims 4 to 6, which is for use in radioimaging a body tumor and wherein the metal chelate is a chelate of ¹¹¹In, ⁶⁷Ga, ⁶⁴Cu, ^{99m}Tc, ⁶⁸Ga, ⁶²Zn, ⁶⁷Cu, ¹⁹⁷Hg, ⁹⁷Ru, ⁵⁷Co or ⁵³Co.

9. A system as claimed in any one of the preceding claims, for use in administering the biotinylated compound selectively at the site of a solid tumor, wherein the binding protein is selectively permeable across the walls of the capillaries which supply the tumor.

10. A substance for use in administering a diagnostic or therapeutic agent to an internal target site of a subject by sequentially administering parenterally to the subject
- (a) a biotinylated compound containing the diagnostic or therapeutic agent,
 - (b) an avidin-containing binding protein capable of localizing selectively at the target tissue when administered to the subject parenterally, and
 - (c) a clearing agent capable of reacting with the binding protein when circulating in the bloodstream of the subject to form a macromolecular aggregate which is cleared by the subject's reticuloendothelial system, said substance being the biotinylated compound (a), the protein (b) or the clearing agent (c).

Claims for the following contracting states: Austria, Spain and Greece

1. A method of preparing a system for administering a diagnostic or therapeutic agent to an internal target site of a subject, the method comprising:
- (a) derivatizing a compound comprising, or for containing, the agent with biotin and forming a biotinylated compound containing the agent;
 - (b) derivatizing a protein comprising avidin with an antibody or antibody fragment to form a binding protein capable of localizing selectively at the target tissue when administered to the subject parenterally;
 - (c) derivatizing a human protein with biotin to form a clearing agent capable of reacting with the binding protein when circulating in the bloodstream of the subject to form a macromolecular aggregate which is cleared by the subject's reticuloendothelial system;
 - (d) providing the products of steps (a), (b) and (c) as said system.
2. A method as claimed in claim 1 wherein the protein comprising avidin is avidin or streptavidin.
3. A method as claimed in claim 1 or claim 2, wherein the biotinylated compound containing the agent is a biotinylated chelate compound complexed with a metal ion to form a stable metal chelate complex.
4. A method as claimed in claim 3 wherein the chelate compound is a metal chelate of 1-phenyl or 1-benzyl EDTA.
5. A method as claimed in claim 4 wherein the epitope-chelate compound is a metal chelate having a thiobutane spacer arm linked to the benzyl moiety.
6. A method as claimed in any one of claims 3 to 5 wherein the metal chelate is a chelate of:
- (a) ^{90}Y , ^{197}Hg or ^{67}Cu , or
 - (b) ^{111}In , ^{67}Ga , ^{64}Cu , $^{99\text{m}}\text{Tc}$, ^{68}Ga , ^{62}Zn , ^{67}Cu , ^{197}Hg , ^{97}Ru , ^{57}Co or ^{53}Co , or
 - (c) iron, copper or ruthenium.
7. A method as claimed in any one of the preceding claims, wherein the binding protein is selectively permeable across walls of tumor-supplying capillaries.
8. A method of preparing a system for administering a diagnostic or therapeutic agent to an internal target site of a subject, the method comprising:
- (a) reacting the N-hydroxysuccinimide ester of biotin (biotin-NHS) with an amine group in a compound comprising, or for containing, the agent to form a biotinylated compound and, if necessary, combining the biotinylated compound with the agent;
 - (b) condensing or coupling an antibody or antibody fragment with avidin or streptavidin to form a binding protein capable of localising selectively at the target tissue when administered to the subject parenterally;
 - (c) reacting a human protein with biotin-NHS or condensing a human protein with biotin to form a clearing agent capable of reacting with the binding protein when circulating in the bloodstream of the subject to form a macromolecular aggregate which is cleared by the subject's reticuloendothelial system; and
 - (d) providing the products of steps (a), (b) and (c) as a kit.
9. (a) A method as claimed in claim 8 wherein in step (a) the compound reacted with biotin-NHS is an EDTA derivative, especially a compound of the formula



- (b) A method as claimed in claim 8 or claim 9(a) wherein in step (b) the antibody or antibody fragment is condensed or coupled with avidin or streptavidin using 2-imminothiolane and succinimidyl 4-(N-mal-

eimidomethyl)cyclohexane-1-carboxylate as cross-linking reagents or a soluble carbodiimide or di-N-hydroxysuccinimide compound as coupling agent,

(c) a method as claimed in claim 8, claim 9(a) or Claim 9(b) wherein the human protein is human transferrin.

10. A modification of a method as claimed in any one of claims 1, 3 to 6 when dependent on claim 1, 8 or 9, wherein step (b) comprises providing avidin or streptavidin as binding protein.

11. (A) A method of preparing a substance for use in administering a diagnostic of therapeutic agent to an internal target site of a subject by sequentially administering parenterally to the patient:

(a) a biotinylated compound containing the agent derivatised with biotin,

(b) an avidin-containing binding protein capable of localising selectively at the target tissue when administered to the subject, and

(c) a clearing agent capable of reacting with the binding protein when circulating in the bloodstream of the subject to form a macromolecular aggregate which is cleared by the subject's reticuloendothelial system, the method comprising:

(i) performing a method as defined in any one of claims 1(a), 3 to 6, 8a or 9a to prepare the biotinylated compound containing the agent derivatised with biotin, or

(ii) performing a method as defined in any one of claims 1(b), 2, 7, 8b or 9b to prepare the avidin-containing binding protein, or

(iii) performing a method as defined in any one of claims 1(c), 8(c) or 9(c) to prepare the clearing agent; or

(B) the use of a method defined in (i), (ii) or (iii) above for preparing, respectively, the resultant biotinylated compound, binding protein or clearing agent for use in sequentially administering parenterally to the patient substances (a), (b) and (c) above.

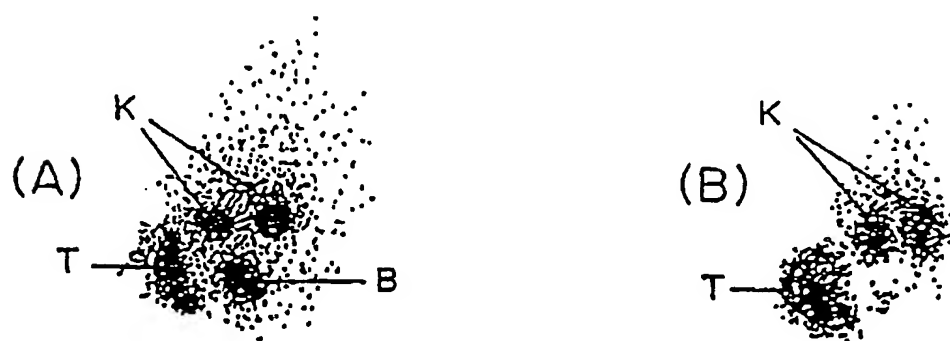
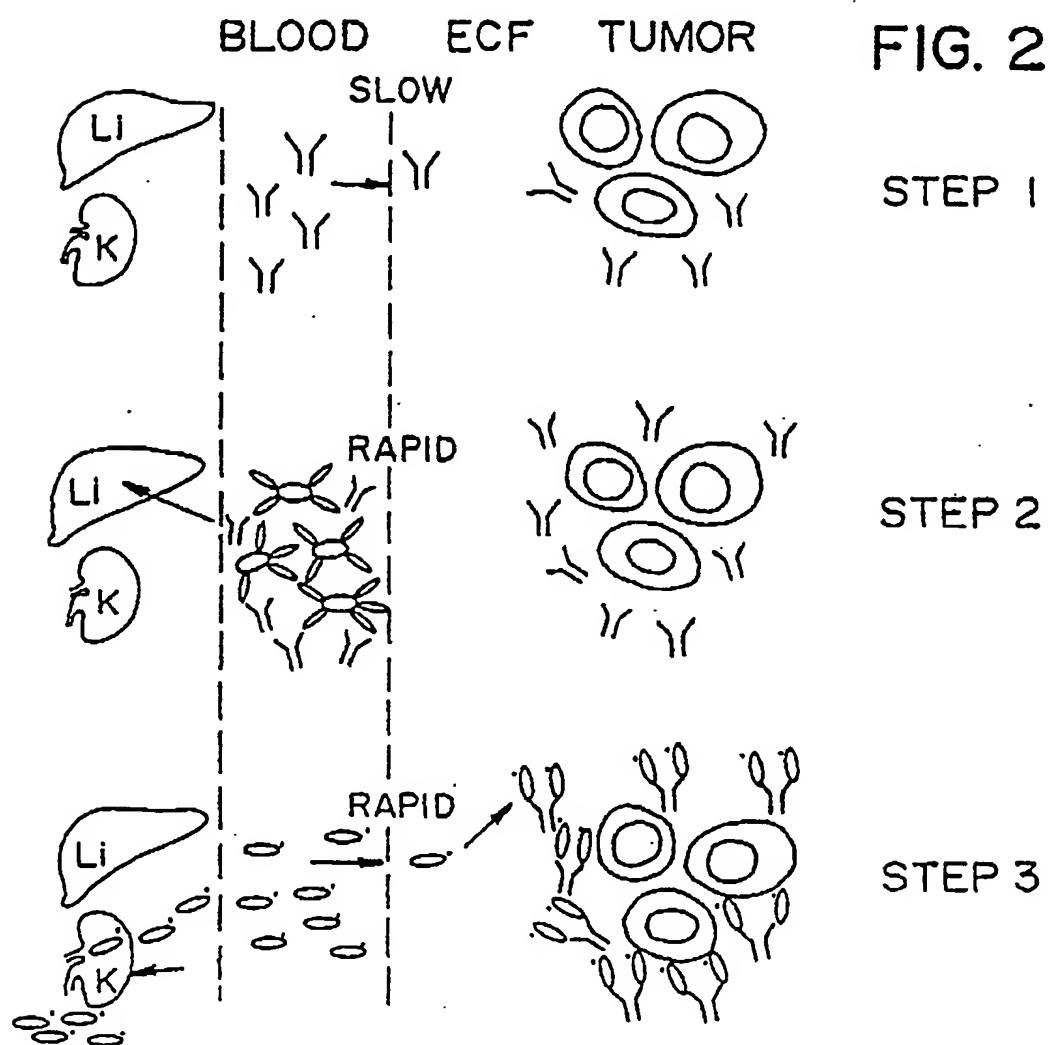
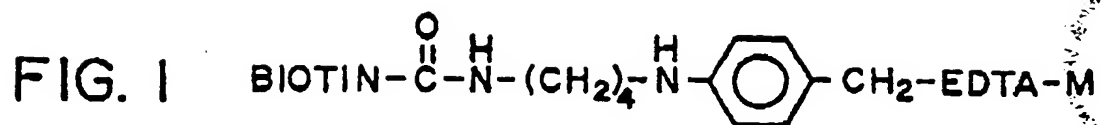


FIG. 3